## REMARKS

Applicants thank the Examiner for telephoning applicants' undersigned attorney Ying Li on June 18, 2003 to indicate that claims 26-32, 34, 46, and 54-57 are allowable. Applicants further thank the Examiner for withdrawing the finality of the previous (July 3, 2002) Office Action.

Applicants have cancelled claim 35 without prejudice. As such, claims 26-32, 34, 36-41, 46, and 52-57 are pending in this application. Of these, claims 26-32, 34, 46, and 54-57 have been amended to claim an influenza vaccine, instead of an influenza antigen. Claim 41 is amended accordingly to reflect that change in base claim 26. In view of the Examiner's comments (see discussions below), base claims 26, 41, and 46 have been further amended to specify that the influenza vaccine is a human vaccine. Also, claims 36-40, 52, and 53 are amended to depend from claim 26, instead of claim 35, which has been now cancelled. Support for these amendments appears throughout the specification, including the claims as originally filed. Thus, no new matter is introduced.

The only remaining issue in this application is Heinen.[1] Citing Heinen, the Examiner rejects claims 35-41, 52, and 53, all of which are directed to an influenza vaccine or a method of making thereof. The Examiner's arguments can be summarized as follows: Heinen shows that M2eHBc did not provide protection against influenza infection in pigs, and quite on the contrary, it exacerbated flu symptoms in pigs; since pigs are a better model for flu vaccine testing than mice, Heinen is evidence that applicants' claimed vaccine would not be

[1] Heinen et al., Journal of General Virology 83:1851-9 (2002).

efficacious. On this ground, the Examiner contends that applicant's disclosure is not enabling for the vaccine claims. 35 U.S.C. § 112, 1st ¶.

Applicants respectfully traverse. In the following, applicants first advance that M2eHBc did not exacerbate flu symptoms in pigs. Applicants then discuss why M2eHBc would not have been expected to provide protection against influenza infection in pigs. Last, applicants show that mice are a widely accepted animal model for flu vaccine testing.

**1. M2eHBc Did Not Exacerbate Flu Symptoms in Pigs**

Heinen vaccinated pigs with a fusion protein, M2eHBc. According to the Examiner, the single experiment described in Heinen included four groups of pigs: (1) pigs receiving M2eHBc alone; (2) pigs receiving M2eHBc plus adjuvant; (3) pigs receiving a plasmid expressing M2eNP; and (4) pigs receiving an empty plasmid.[2] The Examiner argues that group 4 was a proper negative control for the experiment, and that vaccinated pigs (groups 1 and 2) showed more severe clinical signs than the group 4 pigs.[3] See Office Action, p. 9, 3rd ¶. Applicants traverse.

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[2] For clarity, applicants' *Second Supplemental Response to July 3, 2002 Office Action* ("*Second Supplemental Response*") discussed Heinen's data in two parts – the first part related to an experiment in which the animals received a protein vaccine, and the second part related to an experiment in which the animals received a DNA vaccine. The Examiner argues that Heinen describes one, not two, experiment. Whether Heinen describes one or two experiments is a matter of semantics. What is important is that protein vaccination and DNA vaccination are different and the two should be discussed separately. See also Office Action, sentence bridging pp. 12-13.

[3] The Examiner admits that the group 3 results are not relevant because those pigs were administered a DNA vaccine, not a protein vaccine (*supra*). Thus, data with regard to group 3 are not discussed herein.

**A. The Control**

Group 4 was not a proper negative control for the protein vaccination experiment. Applicants agree that an empty DNA plasmid would have been a proper negative control for a DNA vaccination experiment. However, at issue here is protein, not DNA, vaccination. A proper control group should have differed from the experimental groups in only one aspect: the control group should have received only the buffer in which M2eHBc was administered, with or without adjuvant. Heinen's so-called control group contained a DNA plasmid, instead of just a buffer or a buffer plus adjuvant. It is true that Figs. 3 and 4 show the absence of anti-M2e and anti-NP IgG and the lack of lymphoproliferation in group 4 prior to viral challenge (Office Action, ¶ bridging pp. 9-10). But it is utterly unclear whether any components in the DNA preparation had altered the pigs' immune system in a way that was not examined in Figs. 3 and 4. In other words, using a DNA plasmid preparation as control here was just as good as using, say, Coca Cola, as control. And this is just not how a proper experiment is done.

The empty plasmid group was not a proper control also because the empty plasmid was administered differently from the protein vaccines. The empty plasmid was injected intradermally into the pigs, whereas the protein vaccines were injected intramuscularly (Heinen, p. 1853, left col., penultimate ¶). It is unclear how this difference in the route of administration might have contributed to the outcome of the experiment.

**B. The Clinical Data**

Even assuming, *arguendo*, that group 4 was a proper negative control, Heinen's data still fail to show that pigs vaccinated with M2eHBc (groups 1 and 2) did worse than the control pigs. Heinen presents three types of "clinical" data: clinical signs, temperatures, and virus excretion (Figs. 2A-C, respectively). Each of these data types is addressed individually below.

**i. Temperatures**

It is common knowledge that fever is a major symptom of influenza infection. After pooling data from several major studies, Monto[4] concludes that "fever was particularly significant, both as a marker of severity and as an indicator of benefit from treatment" (emphasis added). Thus, body temperature is an important parameter to consider in determining effectiveness of a flu vaccine.

As acknowledged by the Examiner, Heinen shows that the control (group 4) pigs had the highest body temperature. According to Fig. 2B, the mean body temperature of group 4 was statistically higher than that of group 2 (M2eHBc plus adjuvant) on days 1.5, 2, 5, and 7, and statistically higher than that of group 1 (M2eHBc alone) on day 7.[5] These data demonstrate that pigs vaccinated with M2eHBc did better in terms of body temperatures than

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[4] Monto et al., "Clinical signs and symptoms predicting influenza infection," Arch Intern Med 160:3243-7 (2000). The Abstract is attached hereto as Exhibit 1. Applicants will furnish a copy of the full reference upon request by the Examiner.

[5] Where there is a statistically significant difference between group 1 (M2eHBc alone; solid triangle) and group 4 (open square), the data point is indicated by an "a" on top of the graph. Where there is a statistically significant difference between group 2 (M2eHBc plus adjuvant; solid diamond) and group 4, the data point is indicated by a "b." See Fig. 2's legend, last two sentences.

the control pigs; and that among the vaccinated pigs, those vaccinated with M2eHBc plus adjuvant (anti-M2e titre of 2000) did better than those vaccinated with M2eHBc alone (anti-M2e titre of 20). Thus, Heinen's own data, even if taken at their face value, would suggest that M2eHBc immunity provided protection in swine and that a high anti-M2e titre correlated with better protection.

**ii. "Clinical Signs"**

Heinen uses a second clinical indicator in the experiment – the so-called "clinical score" or "clinical signs," which included labored breathing, abdominal breathing, anorexia, apathy and coughing (p. 1854, left col., last full paragraph). Of all five "clinical signs," only coughing is considered a reliable predicator of influenza infection. See, e.g., Monto (Exhibit 1), which states: "The best multivariate predicators of [human] influenza infections were cough and fever . . . ." This statement should also be applicable to swine if, as the Examiner herself states, pigs and humans indeed experience similar influenza symptomology (Office Action, p. 13, 2nd full ¶). Further, unlike body temperatures, which can be measured accurately and objectively, this second indicator is highly subjective.[6] And the authors do not indicate that the measurements of these various "clinical signs" were done in a blinded manner.

In sum, Heinen's "clinical signs" are overall not a reliable indicator for flu vaccine studies. In fact, Heinen itself admits that the "clinical signs" of the animals did not

[6] In contrast, applicants' working examples utilized unambiguous, quantifiable parameters to assess protection, like mortality and body temperatures.

correlate with the animals' temperatures, which, as discussed above, are a more reliable and easily measurable parameter for influenza infection. Thus, Heinen's assertion that M2eHBc exacerbated the "clinical signs" of the vaccinated pigs should be disregarded.

**iii. Viral Excretion**

Heinen states that there was no statistically significant difference in viral excretion between the experimental groups and the control group. Thus, these data do not support that M2eHBc exacerbated flu symptoms in pigs.

**iv. Summary**

Heinen's data should be discounted for lack of a proper negative control. In particularly, Heinen's statement about exacerbated "clinical signs" should be completely disregarded because those "signs" as a whole are not a reliable indicator of vaccine efficacy.

**2. Heinen's M2eHBc Would Not Be Expected to Provide Heterologous Protection**

Applicants pointed out in their *Second Supplemental Response* that Heinen's M2eHBc fusion protein would not have been expected to provide protection against swine influenza infection, because the fusion protein contained a human M2e, which is 26% divergent in sequence from the swine M2e. A tenet of applicants' discovery is that M2e is highly conserved within a single animal species. Applicants do not expect that a 26% divergence is considered "highly conserved" and immunity against one M2e can provide protection against an influenza virus with a drastically different M2e.

The Examiner argues that "the claims do not require that the M2e portion of the fusion protein be species-specific to the animal administered the vaccine" (Office Action, p. 12,

lines 2-3). In view of this comment, applicants have amended base claims 26, 41, and 46 to recite "human influenza vaccine." The remaining base claim, claim 54, already recites an "influenza vaccine for an animal species" and requires that the M2e portion (or an equivalent thereof) be from "said animal species."

The Examiner further argues that "Heinen et al. point out that antibodies induced by immunization bound to the amino acid sequence of the swine influenza challenge virus, indicating that the antibodies induced upon administration of the fusion protein are not species-specific" (Office Action, p. 12, lines 5-8). The Examiner seems to be implying that Heinen's M2eHBc would have been expected to provide protection in pigs because swine antibodies induced by the M2eHBc bound to the swine challenge influenza virus.

This view is incorrect. An antibody population induced by vaccination or by an infection is immensely heterogeneous. There are thousands of, perhaps even millions of, different antibody idiotypes. Heinen does not teach what fraction of the anti-M2e antibody population bound to the swine M2e. Was it 0.1%, or 1 % or 10 % of the population? We do not know. Did it include antibodies responsible for protection? We do not know. Moreover, Heinen mentions in the Methods section that a secondary antibody – "a mAb against swine IgG1" – was used to detect swine IgG1 that had bound to immobilized swine M2e peptide (p. 1853, right col., 4th bullet). This means that only a particular subclass of antibodies was detected. We do not know whether that subclass was representative of the entire anti-M2e antibody population and what fraction this represented relative to the total.

Indeed, Heinen itself does not imply that the cross-reactivity of swine antibodies with a human M2e would have been expected to protect protection, for it speculates, right after the statement about cross-reactivity, that "[i]f the sequence difference caused the absence of protection after vaccination, then this would mean that the spectrum of protection conferred by immunization with M2e does not include viruses with the swine influenza virus M2e sequence" (p. 1857, right col., 1st full ¶). This is true. Applicants' invention is not about using a human vaccine to protect swine. It is about using a human vaccine to protect humans.

The Examiner next argues that "pigs are a natural mixing reservoir for new pandemic strains . . . , which does not exclude the possibility of new [human] influenza viruses comprising the swine influenza M2e sequence" (Office Action, p. 12, lines 9-12). This argument is moot in view of the fact that over the past 70 years, the human influenza M2e sequence has barely changed, regardless of the animal origin of the virus. See, e.g., applicants' *November 4, 2002 Response to July 3, 2002 Final Office Action* ("*November 4, 2002 Response*"), pp. 6-7. And applicants' invention relies on this very conservation of the M2e sequence.

**3. The Mouse Model Is Widely Accepted For Influenza Studies**

The Examiner next argues that according to Heinen, pigs are a better model for studying the efficacy of flu vaccines than mice. The Examiner dismisses applicants' previous argument that three commerical human vaccines were first optimized in mice and then in human clinical trials. The Examiner finds that argument unpersuasive because those vaccines do not appear to have any similarity to the instantly claimed vaccines.

The Examiner's position is misplaced. Whether applicants' vaccine has any similarity in composition to those commercial vaccines is irrelevant. What is relevant is that those commercial vaccines, like the claimed vaccines, are all vaccines purported to protect humans against human influenza infection, and that they, like the claimed vaccines, have all been tested in mice, not pigs. The Examiner presents no scientific evidence showing that the validity of the mouse model for testing one flu vaccine does not justify the validity of this same model for testing a different flu vaccine.

Applicants submitted 48 references in their *November 4, 2002 Response*, demonstrating that mice are an overwhelmingly acceptable animal model to study human influenza, despite the Heinen statement to the contrary. In fact, Field's *Virology*, a well-recognized virology treatise, states: "Mice are an ideal animal model to study pathogenesis and the immune response to influenza viruses . . . ."[7]

## CONCLUSION

Applicants respectfully submit that the application as amended is in condition for allowance. To expedite prosecution, the Examiner is invited to telephone the undersigned

[7] Fields, Virology, 4th Ed., p. 1541 (Exhibit 2).

to discuss any issues remaining in this application.

Respectfully submitted,

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